Final Technical Report

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Center Name: NYU-EPA PM Center: Health Risks of PM Components
Center Director: Morton Lippmann
Title: Immunomodulation by PM: Role of Metal Composition and Pulmonary Phagocyte Iron Status
Investigator: M.D. Cohen
Institution: New York University School of Medicine
EPA Project Officer: Stacey Katz/Gail Robarge
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RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project: Particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) has been shown to induce/exacerbate infectious lung disease and alter the manner by which lungs handle bacteria. This research project sought to validate the hypotheses that: 1) $PM_{2.5}$ modulates lung phagocyte antibacterial function by altering cellular iron (Fe) status; 2) metals (rather than organics, biomatter) in $PM_{2.5}$ underlay any change in lung leukocyte Fe status; and 3) relative Fe content in $PM_{2.5}$ governs these effects. With respect to the latter, in entrained $PM_{2.5}$ with a high relative Fe content, phagocyte uptake and subsequent dissolution of any associated insoluble Fe—combined with an increase in cellular deposition of any soluble Fe (via transferrin [Tf] activity)—will lead to Fe overload and decreased antibacterial function. Conversely, with low relative Fe content-$PM_{2.5}$, presence of relatively greater levels (with respect to Fe) of competitors for Tf binding (e.g., aluminum [Al], manganese [Mn], and vanadium [V]) will bring about Fe deficit and reduced antibacterial function due to inhibited transport of endogenous Fe to cells.

To test the hypotheses, the objectives were: 1) in cooperation with the Los Angeles and Seattle PM Centers, to collect daily $PM_{2.5}$ samples in each metropolitan region over a 3-mo period to characterize patterns of proportionality of Fe to Al, Mn, and V; 2) to determine *in vitro* if a presence of Al, Mn, and V impacted on Fe homeostasis in a rat lung macrophage cell line (i.e., NR8383) when varying doses of each metal (reflective of relative amounts in each city's $PM_{2.5}$) were used; and 3) to examine effects from each city's $PM_{2.5}$ on lung Tf status, Fe status, and antibacterial function of their local macrophages.

Summary of Findings:

Technical Aspects

Daily regional $PM_{2.5}$ samples in New York City (NYC), Los Angeles (LA), and Seattle were collected and analyzed by XRF to determine elemental composition and, specifically, the relative and absolute Fe, Al, Mn, and V contents. Results indicated that: the $PM_{2.5}$ in each city had disparate metal compositions; there were wide variations in absolute and relative Fe, Mn, Al, and

V content; and, there were significant differences in the relative ratios of each competitor to Fe. Based on these differences, *in vitro* studies with NR8383 cells sought to characterize if each competitor (at levels that would be encountered in a given day's $PM_{2.5}$) could alter cell Fe homeostasis and, ultimately, which competitor was most potent in inducing the effect.

Our initial studies using induction of iron response protein (IRP) binding to iron response element (IRE) sequences as an indicator of shift in cellular iron balance indicated that if cells were treated with Fe (as Fe^{3+}) alone or with V, Al, or Mn (individually or in combinations) at levels equivalent to those expected in 500 µg of a given $PM_{2.5}$ sample, each competitor caused Fe deficit in the cells. By employing increasing molar ratios of competitor to Fe, the determination was made that V had the greatest effect on Fe status and Mn the least. Studies using combinations of two or all three competitors indicated that there was a synergistic effect when V and Mn were both present; co-presence of Al with Mn or V had little impact.

To determine if effects observed with varying molar ratios of Al, Mn, and V would reflect what might be occurring with actual $PM_{2.5}$ in the three cities, IRP studies were performed using cells treated with Fe alone and with Al, Mn, and V at levels that would be present in a 500 µg sample of a given day's $PM_{2.5}$. Using treatments that were based on the $PM_{2.5}$ of three randomly-selected days in each city, it was found that levels of IRP activation (compared to that obtained with Fe alone) were greatest in cells treated with the combination of Fe + V + Al + Mn that would be found in NYC. Effects from co-treatments using levels of the metals found in $PM_{2.5}$ from Seattle or Los Angeles were minimal.

Because a presence of nitric oxide (NO) might affect the levels of IRP activation assayed, studies were done to assess inducible nitric oxide synthase (iNOS) levels in the cells. Analyses of ERK-1 and -2 activation were performed concurrently as these MAP kinases are believed to play a role in iNOS formation. Only increasing amounts of Al had significant effects on iNOS expression; treatment with increasing molar ratios of V and Mn failed to induce iNOS to levels significantly above that of Fe alone and below that of iron chelating desferroxamine (DFX). This would suggest that the observed effects from V on IRP activity were unadulterated in that there was no significant increase in NO levels that could enhance IRP-1 binding activity. Results of the ERK studies indicated that increasing molar ratios of V and Al both caused significant increases in phosphorylation (and so, activation) of ERK-1 (p44); only V appeared to increase ERK-2 (p42) activation. Studies to better discern the meaning of these three sets of observations (i.e., changes in IRP activity, iNOS expression, and ERK-1/2 activation) are needed. For now, the results clearly indicate that at least two of these PM-associated metals induce effects on cell Fe homeostasis regulatory mechanisms (i.e., the IRPs—in either a direct or indirect manner) even when there is a level of Fe present that should keep the cell Fe-sufficient.

In light of these results, a re-examination of the three city IRP studies indicated that selected days for Los Angeles had relatively high Al:Fe molar ratios (i.e., > 3.0). As these values fell into the range predicted to cause significant iNOS induction in the NR8383 cells, it is possible that any expected IRP activation was masked by increased NO formation. In contrast, NYC samples had Al:Fe, Mn:Fe, and V:Fe molar ratios that routinely fell into the previously-determined optimal ranges (e.g., 0.75-1.50, 0.04-0.08, and 0.1-0.2, respectively) for inducing enhanced IRP activity in the cells. Samples from Seattle tended to have fairly low V:Fe molar ratios even

while having values for Al:Fe and Mn:Fe expected to induce IRP activation. From these results, and the previous observations on IRP activation using varying molar ratios of these competitors for Tf binding, we concluded that it is the relative amounts of V to that of Fe that are most critical in determining whether a given PM sample is likely to modify the Fe status of a lung macrophage. Furthermore, in PM that contain moderate-to-high amounts of Al, while effects on Fe status are likely, use of the IRP marker as an indicator of this outcome is not practical due to confounding effects introduced by effects on NO formation induced by Al ions.

Performing the *in vivo* exposure studies outlined for Aim 3 was ultimately not possible due to the limitations in the total amount of any given day's sample of $PM_{2.5}$. Instead, the information expected to be gleaned from those studies was obtained, in part, from a concurrent National Institutes of Health/National Institute for General Medical Sciences (NIH/NIGMS)-funded study. Rats were exposed 5 hr/d for 5 d to atmospheres containing physico-chemically distinct forms of V (or other $PM_{2.5}$ metals) and their lung fluids were then analyzed for total Fe content, ferritin and Tf levels. Antibacterial activity in the lungs of exposed cohorts, reflecting the functional status of local macrophages, was also examined. These studies indicated that prior to the start of a lung infection, exposure to pentavalent V—the most common form found in PM—caused significant increases in lavage fluid Fe and ferritin levels, but had less overall effect on total Tf levels. Effects from soluble V were greater than those from an insoluble counterpart. These same result patterns were seen in the ability of the exposed rats to clear a viable bacterial challenge from their lungs, i.e., rats that inhaled soluble V had the most significantly reduced resistance against a pathogen as compared to controls.

These rat study results, taken together with those of the *in vitro* studies performed here, suggest that soluble V ion-induced alterations in the ability of Tf to bind Fe can lead to increases in the levels of free Fe in the airways and, concurrently, less Fe delivery to resident phagocytes. With both more Fe available for sustenance, and local immune cells less capable of performing their normal sentinel duties, the survival of most common bacterial pathogens that invade the lungs would then be greatly enhanced. The specific mechanisms hypothesized and then validated in these studies now allow us to better explain the means by which $PM_{2.5}$—and more importantly, its specific constituents—act to induce or exacerbate infectious lung diseases in exposed populations.

Conclusions

These studies showed that:

- Select metals within a given sample of $PM_{2.5}$ can cause altered cellular Fe homeostasis.

- The effects of $PM_{2.5}$ with respect to altered macrophage Fe homeostasis—from region to region, or site to site in a given region—are governed by the relative content relationships between Fe and at least three co-constituent metals, e.g., V, Mn, and Al. Of these three modulants, V is the most potent effector on this parameter.

- Analysis of IRP activity can be an effective way to examine effects of a wide variety of criteria pollutants upon iron homeostasis in the lungs. But, investigators need to monitor for

effects on NO formation by the pollutants to determine if their measured effects on IRP are being adulterated.

Except when may have been indicated above, the project was found to be technically feasible to conduct.

Supplemental Keywords: NA

Relevant Web Sites: http://www.med.nyu.edu/environmental/
http://es.epa.gov/ncer/science/pm/centers.html